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Effect of Chlorine Position on the Degradation of Dichlorophenols in Anaerobic Pond Sediment

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EFFECT OF CHLORINE POSITION ON THE DEGRADATION OF DICHLOROPHENOLS IN ANAEROBIC POND SEDIMENT

ABSTRACT

To better understand the microbial degradation of chemicals in anaerobic environments, the degradation of chlorinated phenols was examined in freshwater sediments. The study demonstrated that the relative persistence of the dichlorophenols depended on the chemical structure of the individual isomers. The initial step in degradation was the reductive dechlorination of a single chlorine to form the monochlorophenol as an intermediate. Chlorine at the 2-position of dichlorophenols was the most readily reduced. The results show that caution should be used in extrapolating laboratory data to predict degradation rates at field sites.

Introduction.

Chlorinated phenols can reach the environment from accidental release of stored industrial products or wastes (1,2,3), through direct use of products such as pesticides or wood treating compounds (4), or through chlorination of waste treatment effluents containing phenols (5). In some cases, the contamination is severe enough to require remedial action to renovate the contaminated site (6). Current biological remediation methods involve enhancing the conditions for indigenous bacteria to degrade the contaminating compounds (6). An understanding of the potential of the affected environment to degrade the contaminating compounds is needed, however, before such remediation methods can be efficiently used.

Once released to the environment, chlorinated phenols will be distributed between aerobic and anaerobic zones. To adequately establish the environmental fate of these compounds, a clear understanding of their microbiological fate in each zone is required. The fate of these compounds in aerobic environments has been extensively investigated (7,8) whereas the fate of these compounds in anaerobic environments has not received the same level of attention. Fortunately, several researchers have recently initiated such anaerobic studies, and a number of reports (9-16) have appeared that provide some initial insights into the anaerobic degradation of chlorinated phenols. Unfortunately, the majority of these studies have used anaerobic digestor sludge rather than anaerobic sediment or subsurface materials (9,11-16) as the source of microbial activity. Gibson

and Suflita (13) have suggested that results obtained from sewage sludge are not always representative of what occurs in the environment.

To better understand the microbiological degradation of chlorinated phenols in anaerobic environments, we have initiated a program to investigate the degradation of these compounds in anaerobic freshwater sediments. We report here the results of several studies to determine (a) whether the position of chlorine substituents on a series of dichlorinated phenols affected the rate of degradation of these compounds, (b) whether stable degradation intermediates would be produced during the degradation process, and (c) whether the dechlorination step was primarily a microbiological reaction or a chemical reaction.

Persistence of Dichlorophenol Isomers.

The relative persistence of 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-dichlorophenol was examined in five freshwater sediments collected in the vicinity of Athens, GA. A sample of these results is shown in Table 1. The 2,3- and 2,4- isomers were generally degraded (lost from the slurry) within 2 to 4 weeks of incubation. Similar rapid rates of degradation were observed in all five sediments. The results for 2,4-dichlorophenol are shown in Table 2. The remaining four isomers generally persisted for the duration of a given experiment, which would last from 12 to 14 weeks. This persistence was observed in four of the sediment samples tested, an example of these results is shown in Table 3. One sediment sample (Cherokee Trailer Park Sediment, Table 4),

however, exhibited significant degradation of the 2,5-, 2,6-, 3,4- and 3,5-isomers.

Unlike the degradation of numerous chlorinated aliphatic compounds, where both abiotic and biotic processes can be important (17), the degradation of the chlorinated phenols was strictly a microbiological activity. Several observations support this conclusion: (a) degradation products could only be identified in biologically active sediments, (b) significant decreases in dichlorophenol concentration were not observed in autoclaved controls (Table 5), (c) degradation always occurred after a period of acclimation, and (d) repeated addition of dichlorophenol resulted in increased activity (Fig. 1).

With the exception of the 2,5- and 2,6- isomers, our observations in freshwater sediments were in qualitative agreement with the results obtained by Boyd and Shelton (16) who investigated the degradation of dichlorophenols in sewage sludge. In both studies, the 3,4- and 3,5-substituted isomers generally were persistent and the 2,3- and 2,4-isomers were completely lost from solution over the respective incubation intervals. We observed, however, that the 2,5- and 2,6-isomers were relatively persistent whereas Boyd and Shelton (16) found that these isomers were degraded at rates comparable to the 2,3- and 2,4- isomers. Interestingly, we did observe that each of the 2-substituted isomers were degraded at similar rates only in the contaminated Cherokee Trailer Park sediments.

The loss of the more persistent isomers with Cherokee

Trailer Park sediments may have resulted from enriched microbial activity because of site contamination. The only readily apparent differences between the sediments studied was the presence of asphalt in the Cherokee Trailer Park sediments. Sediment samples were taken from a point in the pond adjacent to a newly paved road. When these sediments were sieved at the laboratory, many asphalt-coated rocks were separated from the sediment. It is possible that soluble organic components of the asphalt may have led to the development of an anaerobic community capable of degrading dichlorinated phenols. Similar contamination was not observed at the other sites nor did the five sites differ markedly in pH, organic carbon content, soluble sulfate concentration (Table 6) or in the distribution between bound and soluble forms of the dichlorophenols (Table 7).

Intermediate Product Formation.

Previous studies have indicated that under anaerobic conditions the degradation of chlorinated phenols is initiated by removal of a chloride anion by reductive dechlorination (9,15) to form phenol that is then converted to CO_2 and CH_4. For phenols containing two or more chlorine substituents, the chlorines have been observed to be removed sequentially by the same mechanism (10,11).

We found that reductive dechlorination was the initial step in the degradation of dichlorophenols in the five freshwater sediments. Whenever degradation of a dichlorophenol substrate was observed, the formation of a monochloro-intermediate also was

detected (Table 8). The 2- and 3-chlorophenols, for the most part were transient (2 to 6 weeks) intermediates; however, in some cases (Fig. 2) they persisted for the duration of the experiment. In contrast, the 4-chlorophenol intermediate was always persistent throughout a given study (Fig. 3).

Phenol, which would be a product of the dechlorination of the monochloro-intermediates in the above studies, was only observed in a few instances. This was anticipated because phenol is readily degraded under anaerobic conditions (10). We were, however, able to identify phenol in experiments (see footnote Fig. 1) designed to acclimate sediments to the degradation of a particular dichlorophenol. Phenol was detected in greatest concentration in acclimated cultures after readdition of the particular dichlorophenol following substrate depletion for several days.

For dichlorinated phenols, chlorines in the 2-position were the most susceptible to reductive dechlorination. The 3-position was intermediate in its susceptibility, and the 4-position was the least likely to be removed. The 2,4- and 2,5-isomers, for example, produced the 4- and 3-chlorophenol products; the 2,3-isomer, which was the only dichlorophenol to yield a mixture of monochloro products, produced the 3-chlorophenol as its major product (Fig. 2) and the 2-chlorophenol as a minor product. The loss of chlorine from the 4-position was only observed in the dechlorination of 3,4-dichlorophenol to 3-chlorophenol and then it was only observed in the Cherokee Trailer Park sediment. The

3,4-isomer was the least degradable of the dichlorophenols.

The degradation of the monochloro intermediates followed the pattern 2 > 3 > 4. The 4-chlorophenol was the most recalcitrant intermediate and was not observed to degrade in all sediments, including Cherokee Trailer Park sediments, over the 12- to 14-week incubation period. Similar results were observed for the monosubstituted phenols by Boyd and Shelton (16); however, they observed complete degradation of the 4-chloro isomer within 6 weeks of its addition to unacclimated sludge.

Effect of Acclimation on Rate of Degradation.

Sediments became acclimated to the degradation of a given dichlorophenol after repeated addition of the dichlorophenol to the sediment slurry. Acclimated cultures prepared with 2,4- and 3,4-dichlorophenol, for example, ultimately reached similar levels of activity for Cherokee Trailer Park sediments (Fig. 1). These isomers represent, respectively, the least and most persistent compounds in unacclimated sediments. Once the cultures were acclimated, disappearance of the compound occurred without a lag period.

Numerous mechanisms have been postulated to explain the acclimation of natural waters and sediments. Although these mechanisms include enzyme induction (18,19), exchange of genetic material (20,21), low levels of inorganic nutrients (22,23), and preferential use of other substrates (22,24), most acclimation phenomena have been attributed, completely or in part, to the time required for small populations of mineralizing organisms to

become large enough to cause a detectable loss in the chemical (25-28). In most situations, an increase in degradative activity resulted in a corresponding increase in the number of bacteria able to mineralize the compound as measured by compound -specific MPN techniques (29). Because in most cases the added compound was mineralized to a significant degree, the increase in the active bacterial population presumably resulted from the organism's ability to use the compound as a source of carbon and energy.

Acclimation is not always a result of growth of the active organisms on the added compound. Interestingly, the acclimation of freshwater sediments to the anaerobic degradation of 2,4-dichlorophenol (Fig. 1) occurred without significant mineralization of the 2,4-dichlorophenol. Following the initial addition and for several subsequent additions, the 2,4-isomer was stoichiometrically converted to 4-chlorophenol, which was not further degraded. Similar results were observed with the 3,4-isomer. Apparently the organisms' ability to reductively dechlorinate 2,4- or 3,4-dichlorophenol was a competitive advantage and presumably allowed the population to grow on other organic components of the sediment while using the 2,4- or 3,4-isomer as an electron sink.

Degradation of dichlorinated phenols always was initiated following an acclimation period of at least 2 weeks in our fresh water sediment studies. Boyd and Shelton (16), on the other hand, observed that the 2-substituted isomers were degraded

without a lag or acclimation period in sewage sludge. They suggested that the loss of dichlorophenols may have resulted from nonspecific electron carriers in the unacclimated sludge. An alternative explanation could be that wastes entering the treatment plant may have supported an anaerobic bacterial population able to degrade these compounds. Such a possibility is supported by the enhanced degradation rates and the reduced acclimation periods that we observed in the Cherokee Trailer Park sediments.

Extrapolation to Other Environments.

The decision to use biological treatment as a method for the remediation of contaminated environments generally rests on the availability of information indicating the biological potential for degrading the contaminating compounds. To address this need, numerous biological assays have been developed (13,14,30-32). Unfortunately, they all suffer the same flaw: the results from a given assay study cannot necessarily be used to predict degradation rates in other environments. Gibson and Suflita (13), for example, have suggested that sewage sludge cannot be used as a standard inoculum for biodegradation studies, as had been reported by Shelton and Tiedje (14).

Our results support Gibson and Suflita's contention. We observed that only two of six dichlorophenols were degraded readily in uncontaminated freshwater sediments, whereas Boyd and Shelton (16) observed degradation of four of the six isomers. We always observed a lag period before degradation occurred. Boyd and

Shelton (16) reported immediate degradation without an acclimation period. It is apparent that using the results from the sewage sludge studies to predict the fate of dichlorophenols in freshwater sediments would have led to many inconsistencies.

Probably, the most appropriate information for deciding on a biotreatment scenario would be data from the contaminated site. This becomes even more apparent when the degradation rates that we observed for the contaminated Cherokee Trailer Park sediment are compared to the uncontaminated sediments. Extrapolation of data from uncontaminated sediments to contaminated sediments would have predicted much slower rates of degradation than were observed, whereas extrapolation from contaminated to uncontaminated would have predicted much faster rates than were determined.

Summary.

We would like to emphasize several points. The relative persistence of the dichlorophenols in anaerobic sediments depended on the chemical structure of the individual isomers. The initial step in degradation was the reductive dechlorination of a single chlorine to form the monochlorophenol as an intermediate. Chlorine at the 2-position of dichlorophenols was the most readily reduced. Finally, caution should be used in the extrapolation of laboratory data to predict degradation rates at field sites.

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Table 1. Persistence of dichlorophenol isomers in freshwater sediment slurries: Bolton's Pond.

Isomer b	Initial Conc. (mg/L)	Residual Dichlorophenol Conc. (mg/L) Over Time of Incubation a						
		Week 0	2	4	6	8	10	12
2,3-	4.62	4.62	3.70	1.93	0	0	0	0
		4.48	3.80	0	0	0	0	0
2,4-	4.74	4.61	3.08	0	0	0	0	0
		4.74	2.28	0	0	0	0	0
2,5-	4.96	4.50	4.80	4.60	4.41	4.45	4.09	3.89
		3.99	4.96	4.50	4.55	4.61	4.29	4.02
2,6-	4.17	3.51	3.58	4.17	3.53	3.33	3.33	2.83
		2.97	3.63	4.17	3.72	3.39	3.57	2.83
3,4-	4.45	4.32	4.18	4.12	3.87	4.45	3.79	4.22c
		4.16	4.18	3.81	3.87	4.45	3.44	4.45
3,5-	4.18	3.78	4.18	2.87	0	0	0	0 c
		4.18	4.18	4.18	4.18	4.18	4.18	3.41

a. Sediment (0-5 cm) and overlying water were collected from Bolton's Pond in sterile wide-mouth quart Mason jars, which were filled to capacity and capped near the sediment/water interface. Site water also was collected in sterile Erlenmeyer flasks. Following transport to the laboratory, the samples were placed in an anaerobic chamber with an atmosphere 95% N2:5% H2 and allowed to incubate for several days to remove oxygen residuals. The sediment was washed through a 1-mm sieve with site water to remove stones and organic debris. The sieved sediment was allowed to settle in glass crystallizing dishes (170 x 90 mm) for 1 to 2 months before being used. Sediment slurries, containing 2 g dry weight equivalent of sediment and enough site water to yield a total volume of 20 ml, were prepared in 100-ml serum bottles. Experiments were initiated by the addition of dichlorophenol to a final

concentration of 4 or 11 ppm. At prescribed intervals, 1-ml samples of the slurries were removed from the serum bottles and added to 1 ml of acetonitrile. The combination was vigorously mixed and then centrifuged 10 minutes at 3500 rpm. The supernatant solution (100 uL) was injected on a Dupont 8800 HPLC with an octadecylsilane column and mobile phase of 60:38:2 methanol:water:acetic acid run at 1.5 ml/min. A Micromeritics 788 Dual Variable detector set at 280 nm and a Spectraphysics 4100 integrator were used to detect and quantitate parent compounds and products by comparison of retention times with authentic standards.

b. All chlorophenols were obtained from Aldrich Chemical Company (Milwaukee, Wisconsin).

c. These samples were analyzed at 14 weeks.

Table 2. Persistence of 2,4-dichlorophenol in freshwater sediment slurries.

Sediment	Initial Conc. (mg/L)	Residual 2,4-Dichlorophenol Conc. (mg/L) Over Time of Incubation a						
		Week 0	2	4	6	8	10	12
Bar H	4.94	4.86	4.94	0	0	0	0	0
		4.72	4.62	0	0	0	0	0
Cherokee Trailer Pk.	4.70	4.70	4.45	0	0	0	0	0
		4.70	4.70	0	0	0	0	0
2-Boat Pond	4.79	4.79	4.66	0	0	0	0	0
		4.79	4.79	0	0	0	0	0
Sandy Creek Nature Ctr.	4.84	4.82	3.01	0	0	0	0	0
		4.82	4.17	0	0	0	0	0
Bolton's Pond	4.74	4.61	3.08	0	0	0	0	0
		4.74	2.28	0	0	0	0	0

a. See footnote Table 1. for experimental procedure.

Table 3. Persistence of 3,5-dichlorophenol in freshwater sediment slurries.

Sediment	Initial Conc. (mg/L)	Residual 3,5-Dichlorophenol Conc. (mg/L) Over Time of Incubation a						
		Week 0	2	4	6	8	10	14
Bar H	4.36	3.30	3.48	3.57	4.35	3.01	3.23	2.18
		4.18	4.36	4.36	4.36	4.01	4.14	3.26
Cherokee Trailer Pk.	4.14	4.14	4.14	3.74	0	0	0	0
		4.14	4.14	4.09	0	0	0	0
2-Boat Pond	4.22	4.22	4.22	0	0	0	0	0
		4.22	4.22	4.18	4.12	3.92	4.04	4.06
Sandy Creek Nature Ctr.	4.27	4.27	4.27	4.00	4.27	3.84	4.24	4.27
		4.27	4.27	3.74	4.27	4.01	4.24	3.48
Bolton's Pond	4.18	3.78	4.18	2.87	0	0	0	0
		4.18	4.18	4.18	4.18	4.18	4.18	3.41

a. See footnote Table 1. for experimental procedure.

Table 4. Persistence of dichlorophenol isomers in freshwater sediment slurries: Cherokee Trailer Park.

Isomer	Initial Conc. (mg/L)	Residual Dichlorophenol Conc. (mg/L) Over Time of Incubation a						
	Week	0	2	4	6	8	10	12
2,3-	4.57	4.57	3.60	0	0	0	0	0
		4.57	3.33	0	0	0	0	0
2,4-	4.70	4.70	4.45	0	0	0	0	0
		4.70	4.75	0	0	0	0	0
2,5-	4.91	4.91	3.96	4.30	0	0	0	0
		4.35	4.02	4.20	3.92	0	0	0
2,6-	4.12	2.85	4.02	4.04	0	0	0	0
		3.03	3.25	4.12	3.35	0	0	0
3,4-	4.40	4.32	3.60	4.12	0	0	0	0
		4.40	3.71	4.02	3.98	4.02	3.32	4.02
3,5-	4.14	4.14	4.14	3.74	0	0	0	0
		4.14	4.14	4.09	0	0	0	0

a. See footnote Table 1. for experimental procedure.

Table 5. Persistence of dichlorophenols in autoclaved freshwater sediment slurries.

Isomer	Initial Conc. (mg/L)	Residual Dichlorophenol Conc. (mg/L) Over Time of Incubation a					
	Week	0	2	4	7	10	14
2,3-	4.71	4.71	4.71	4.71	4.71	4.31	3.15
	11.76	11.76	11.76	11.32	11.76	10.33	11.76
2,4-	4.84	4.84	4.84	4.84	4.66	3.52	3.61
	11.78	11.78	11.78	11.78	10.04	9.57	10.18
2,5-	4.07	4.07	4.07	3.89	4.07	4.07	2.04
	11.73	10.74	9.54	7.34	8.04	5.95	3.19
2,6-	4.25	4.25	4.25	4.25	4.25	3.65	3.36
	11.38	11.38	11.38	10.80	11.38	9.66	10.56
3,4-	4.54	4.54	4.54	n.d.	4.54	4.10	4.15
	11.33	10.55	11.14	11.33	10.98	8.40	8.70
3,5-	4.27	4.27	4.27	4.27	4.27	4.27	3.56
	11.41	11.41	11.41	11.41	10.95	10.03	10.06

a. Sediment slurries autoclaved for 30 minutes on each of three successive days in crimp-sealed, 100-ml serum bottles. Except for sterilization, samples were treated in the manner described in Table 1.

Table 6. Sediment physical/chemical characteristics.

Site	pH a	TOC b (%)	Sulfate c (mg/L)
Bar H	7.28 (0.10)d	0.33 (0.02)	16.8 (5.9)
Cherokee Trailer Park	7.09 (0.15)	0.79 (0.06)	12.7 (4.0)
2-Boat Pond	7.07 (0.20)	1.15 (0.13)	27.8 (7.6)
Sandy Creek Nature Center	6.90 (0.06)	1.30 (0.12)	25.2 (2.3)
Bolton's Pond	6.90 (0.21)	0.64 (0.07)	24.8 (4.2)

a. An Orion Ross combination pH electrode was used to determine the pH of sediment slurries containing 2 g dry weight sediment and enough site water to yield a final volume of 20 ml.

b. The organic carbon content of each sediment was determined using an Oceanography International Corporation carbon analyzer. Sediments were dried at 105oC, ground, and weighed into ampoules to which 1 ml 10% v/v H_3PO_4 was added, followed by 2 ml water. After 30 minutes, samples were mixed with 2 ml each of saturated potassium persulfate and water and purged of inorganic carbon with CO_2-free O_2 for at least 6 minutes. The ampoules were sealed and then autoclaved for 30 minutes, and the CO_2 released from the organic matter was identified and quantitated using a Horiba infrared detector and integrator. Reagent blanks and standards (potassium biphthalate) were prepared and analyzed in the same way but without dried sediment.

c. Samples for sulfate analysis were prepared in the following manner.

Wet sediments were first shaken with water (1:5 w/v) for 15 minutes at 200 rpm on a G24 Environmental Incubator Shaker (New Brunswick Scientific Co. Inc.) followed by centrifugation at 3500 rpm for 10 minutes. After supernatant solution removal, the residue was resuspended in 0.05 M Na_2HPO_4 (1:5 w/v) and shaken 30 minutes at 200 rpm. Following centrifugation and supernatant solution removal, the pellet was resuspended in 0.05 M Na_2HPO_4 (1:5 w/v) and treated as previously described. Each of the 3 washes was passed through both a 0.45 and a 0.22 um filter. Sulfate levels were determined with a Dionex 2020i ion chromatograph. A fast run anion column was used with a 0.003 M $KHCO_3$/0.0024 M K_2CO_3 eluent run at 2.5 ml/minute, and a 0.025 N H_2SO_4 solution was used with the continuous flow fiber suppresser. The total sulfate in the 3 washes from each sample was detected and quantitated with an electrometer and a Hewlett-Packard 3390A integrator by comparison with Na_2SO_4 standards prepared in water. The values reported in the Table represent the total of these three fractions.

d. Values in parentheses represent standard error of the mean for triplicate samples.

Table 7. Distribution of dichlorophenols between sediment and solution in freshwater sediment slurries.

	Dichlorophenol Isomers					
	2,3-	2,4-	2,5-	2,6-	3,4-	3,5-
pKa	7.70	7.89	7.51	6.79	8.59	8.19
0 Time						
% Sorbed a	39.79	25.60	18.50	14.66	28.63	27.35
% Soluble	63.21	46.30	58.79	99.10	41.25	66.03
24 Hours						
% Sorbed	30.16	6.44	25.42	9.44	52.42	45.49
% Soluble	58.92	61.49	87.70	93.41	57.47	44.68

a. The distribution of chlorophenol isomers between soluble and bound forms was determined as follows. Each isomer was added individually to 100-ml serum bottles containing a slurry of 2 g dry weight sediment in sufficient anaerobic site water to yield a final volume of 20 ml. At 0 and 24 hours, a 2-ml slurry sample was removed, centrifuged for 10 minutes at 3500 rpm, and the supernatant solution analyzed as described in Table 1. The pellet was resuspended in 2 ml of acetonitrile then centrifuged at 3500 rpm, and the supernatant solution was analyzed for dichlorophenol. The total recoverable chlorophenol was determined by analysis of the supernatant solution from 2 ml of the slurry, which was vigorously mixed with 2 ml of acetonitrile and centrifuged for 10 minutes at 3500 rpm. The percentages of soluble and sorbed chlorophenol were determined by dividing the amount recovered from solution and from the pellet, respectively, by the total chlorophenol recoverable and

multiplying by 100. Adjustments were not made for the water entrained in the pellet fraction.

Table 8. Intermediate products formed during the transformation of dichlorophenols. a

SUBSTRATE	PRODUCT
2,3-DICHLOROPHENOL	3-CHLOROPHENOL 2-CHLOROPHENOL
2,4-DICHLOROPHENOL	4-CHLOROPHENOL
2,5-DICHLOROPHENOL	3-CHLOROPHENOL
2,6-DICHLOROPHENOL	2-CHLOROPHENOL
3,4-DICHLOROPHENOL	3-CHLOROPHENOL
3,5-DICHLOROPHENOL	3-CHLOROPHENOL

a. Products were identified by comparison of retention times with authentic standards and by co-chromatography with same standards.

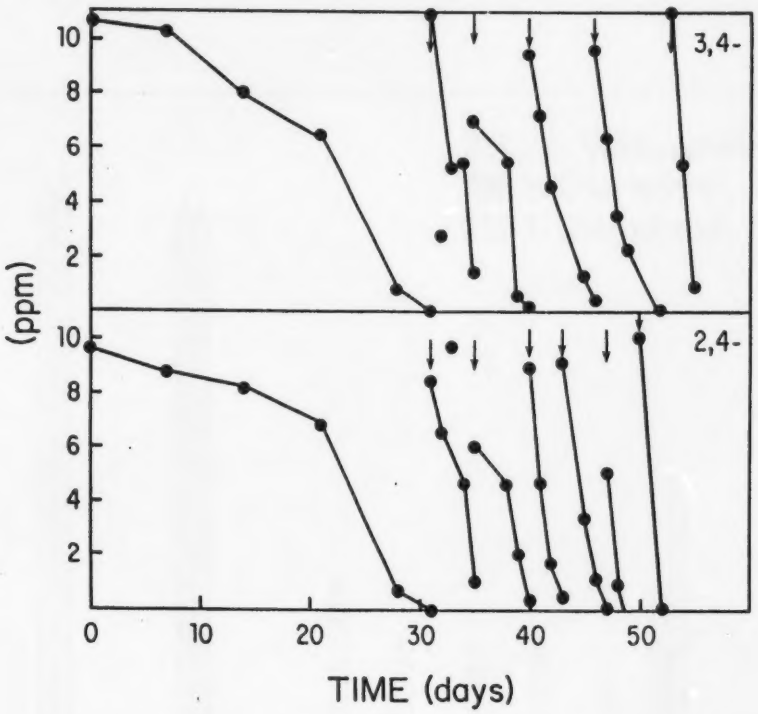

Figure 1. Acclimation of freshwater sediment slurries to the degradation of 2,4- and 3,4-dichlorophenol. a
a. Sediment was collected from Cherokee Trailer Park pond and prepared as described in Table 1. Sediment slurries containing 10 g dry weight sediment and sufficient anaerobic site water to yield a final volume of 100 ml were prepared in 125-ml serum bottles. The 2,4- and 3,4-dichlorophenol standards were prepared in water and added to the slurries to a final concentration of about 11 ppm. At specified intervals, 1 ml of the slurry was removed, vigorously mixed with 1 ml acetonitrile, and centrifuged for 10 minutes at 3500 rpm. The supernatant solution was analyzed as described in Table 1. When the parent compound was no longer detectable, enough water from the site was added back to the serum bottle along with the dichlorophenol standard to give the initial concentration of about 11 ppm in a total volume of 100 ml. This monitoring/respiking procedure was repeated several times in order to observe any changes in the rates at which the isomers could be degraded.

The data points are connected by lines as an aid to the viewer. Points not connected were considered to be the result of experimental error but were shown to complete the data set.

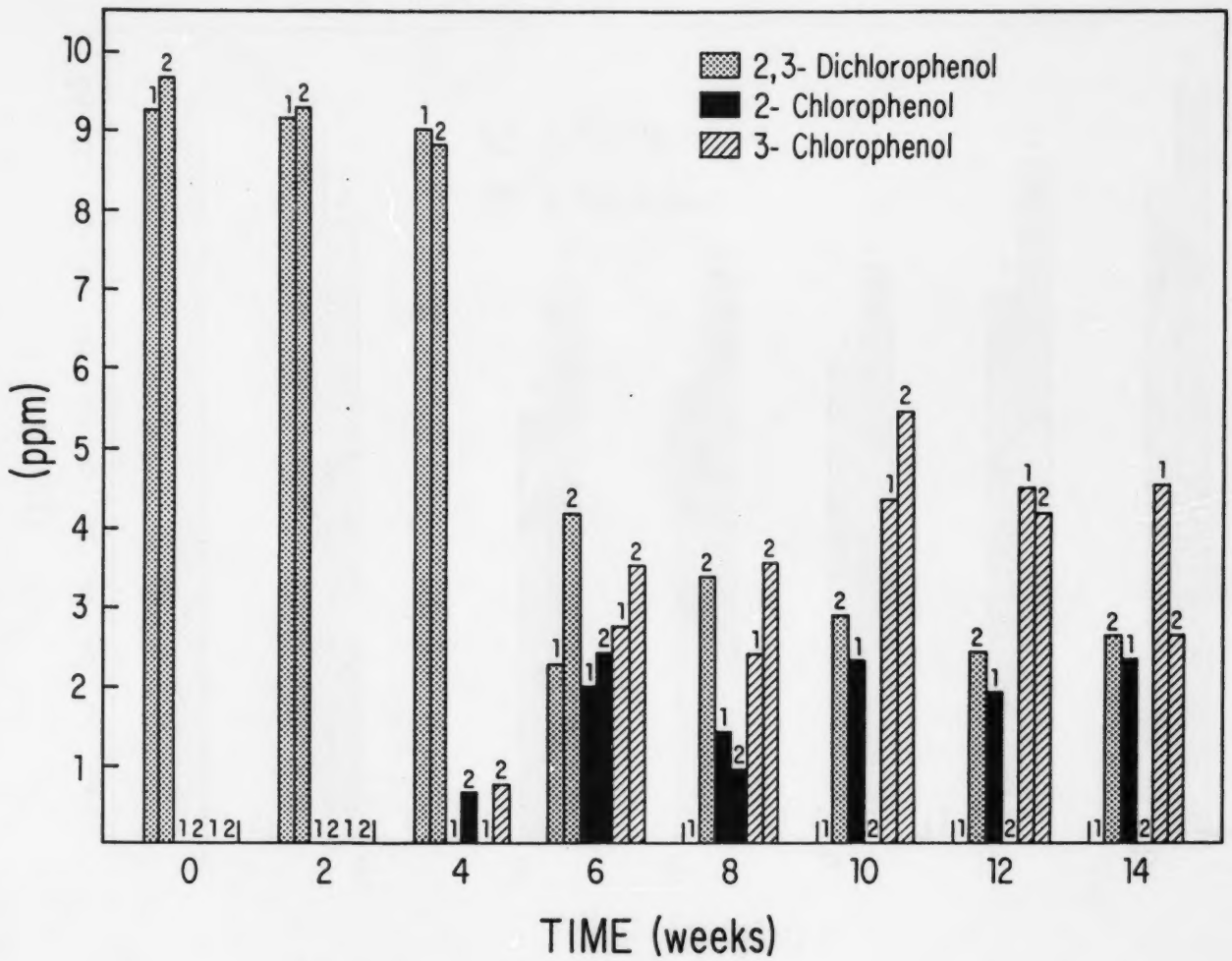

Figure 2. Intermediate product formation during the transformation of 2,3-dichlorophenol in freshwater sediment slurries. a

a. Slurries were analyzed as described in Table 1.

Chlorophenols were detected by comparison of retention times with authentic standards and by co-chromatography of samples with added standards.

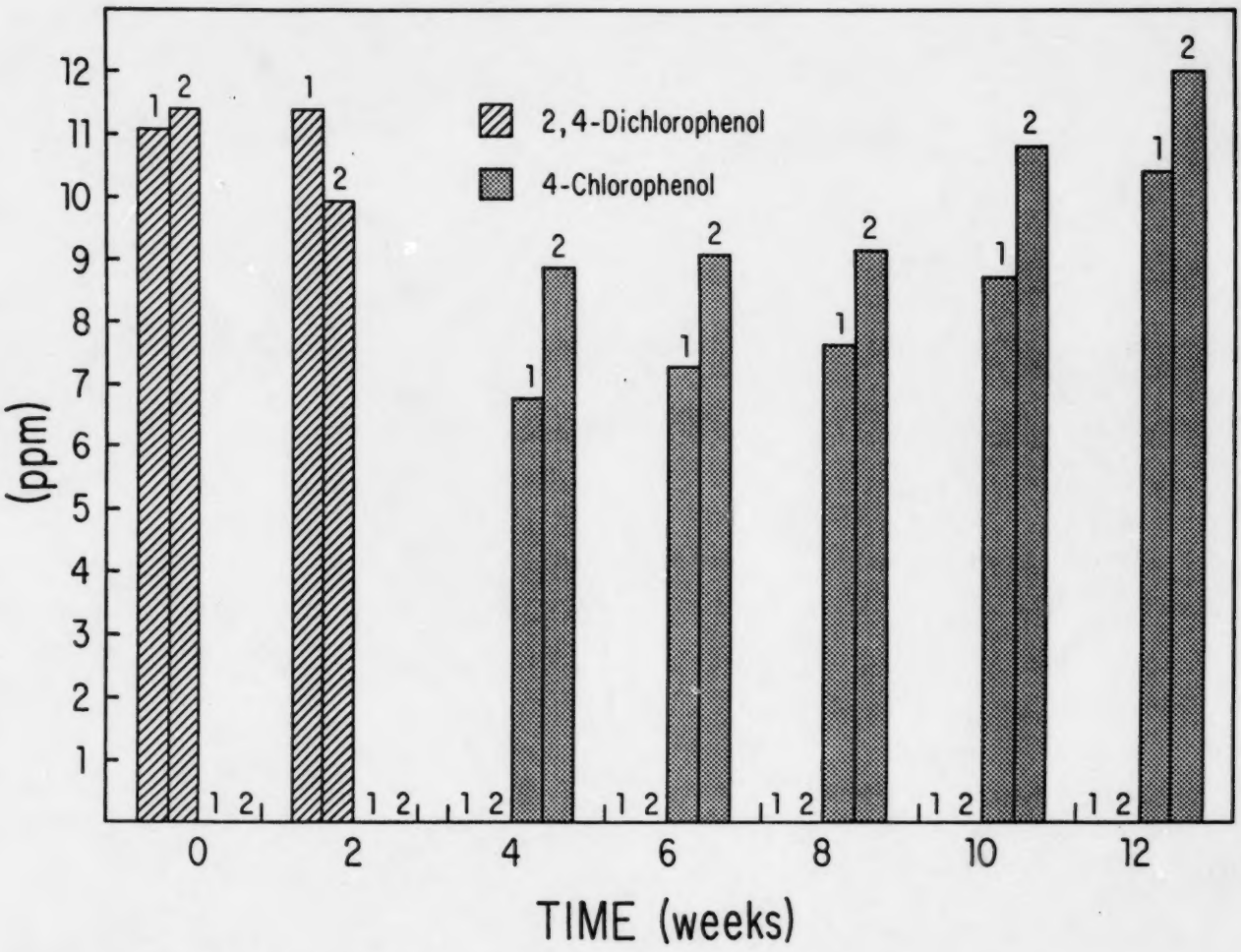

Figure 3. Intermediate product formation during the degradation of 2,4-dichlorophenol in a freshwater sediment slurries. a

a. Slurries were analyzed as described in Table 1. Chlorophenols were detected by comparison of retention times with authentic standards and by co-chromatography of samples with added standards.